see original articles on pages 211 and 232 (77(3), February 2010)

Does the downregulation of the FGF23 signaling pathway in hyperplastic parathyroid glands contribute to refractory secondary hyperparathyroidism in CKD patients?

Marie-Hélène Lafage-Proust[1]

Compared to normal tissue, hyperplastic parathyroid glands of patients with chronic kidney disease on dialysis express lower levels of FGFR1 and Klotho proteins. Similar findings are reported in uremic rats with advanced chronic kidney disease. Moreover, in these animals, FGF23 administration fails to reduce PTH serum levels *in vivo* and to transmit downstream signals in parathyroid cells *ex vivo*. These findings may explain, at least partly, the concomitant elevation of both FGF23 and PTH serum levels in chronic kidney disease secondary hyperparathyroidism.

Kidney International (2010) **77,** 390–392. doi:10.1038/ki.2009.512

Less than 10 years ago, mutations of fibroblast growth factor 23 (*FGF23*) were shown to be responsible for autosomal dominant hypophosphatemic rickets.[1] Ever since, a large body of data has demonstrated that FGF23 is a major actor in phosphate metabolism, and this hormone has therefore drawn tremendous attention from the community of nephrologists. However, the interplay among phosphate, calcium, parathyroid hormone (PTH), vitamin D, and FGF23 in the chronic kidney disease (CKD) context remains not fully understood. At physiological concentrations, FGF23 signals through FGF receptors (FGFRs) 1, 3, and 4 only when bound to Klotho, a transmembrane protein that can also be released from the cell surface and that acts as a co-receptor. Thus, the expression and/or availability of Klotho determine the tissue specificity and, potentially, the amplitude of effects of its ligand, FGF23. The phenotypes of *FGF23*$^{-/-}$ and *Klotho*$^{-/-}$ mice are very similar (high phosphate and calcitriol serum levels and tissue calcifications), suggesting that many characteristics of Klotho deficiency result from a lack of FGF23 action, and constituting further evidence that Klotho controls FGF23 signaling. Klotho has a rather restricted pattern of expression: kidney (distal tubules), parathyroid glands, brain, muscles, gonads, duodenum, and pancreas. The essential role of FGF23 is to regulate renal phosphate handling by enhancing kidney proximal tubule phosphate excretion in urine through the downregulation of the sodium–phosphate cotransporters NPT2a and NPT2c. Matrix-embedded osteocytes, the most abundant bone cells, account for most of the physiological circulating FGF23 levels.[2] In bone, FGF23 synthesis is stimulated by calcitriol, and, in turn, as a negative-feedback loop, FGF23 reduces $1,25(OH)_2$ vitamin D serum concentration via inhibition of 25-hydroxyvitamin D–1α-hydroxylase (CYP27B1) and stimulation of 24-hydroxylase activity (CYP24) in kidney tubules (Figure 1a).[3] Interestingly, disruption of the calcitriol signaling pathway (through vitamin D receptor (VDR) or 25-hydroxyvitamin D–1α-hydroxylase deletion) blunts most of the effects on mineral metabolism and tissue calcifications induced by FGF23 or Klotho deficiency.[4,5]

The relationships between FGF23 and parathyroid glands remain a matter of debate. On the one hand, there is solid evidence that FGF23 downregulates PTH secretion. Parathyroid cells express both FGFR1 and FGFR3, as well as Klotho.[6] Urakawa *et al.* showed that intravenous injection of FGF23 in mice or rats rapidly induces the expression of *egr1*, an early gene downstream of FGFR signaling, in parathyroid glands.[7] Moreover, addition of FGF23 to rat or bovine parathyroid gland cell cultures decreases PTH secretion[6] and mRNA expression[8] in a dose-dependent manner. Finally, in contrast to its effects in the kidney, FGF23 stimulates 25-hydroxyvitamin D–1α-hydroxylase in parathyroid glands, suggesting that it could also indirectly downregulate PTH expression through an increase in the local production of calcitriol. Moreover, Klotho may play an FGF23-independent role in low-calcium-induced PTH release,[9] making things even more complicated.

On the other hand, there are experimental or clinical conditions in which PTH and FGF23 levels can be increased concomitantly, which could be explained in some, but not all, cases by a disruption of the FGF23 signaling pathway. First, mice overexpressing FGF23 exhibit high PTH serum levels with parathyroid gland hyperplasia, which were previously attributed to the concomitant decrease in serum $1,25(OH)_2$ vitamin D.[10] Brownstein *et al.*[11] reported the case of a hypophosphatemic patient with both severe hyperparathyroidism and increased FGF23 serum levels. This patient exhibited high levels of serum Klotho (due to a chromosomal translocation with a breakpoint adjacent to the *Klotho* gene),

[1]*Université de Lyon, Institut National de la Santé et de la Recherche Medical (INSERM) 890, CHU , Saint-Etienne, France*
Correspondence: *Marie Hélène Lafage-Proust, INSERM 890,15 Rue A Paré, 42023 Saint-Etienne cedex 2, France. E-mail: lafagemh@univ-st-etienne.fr*

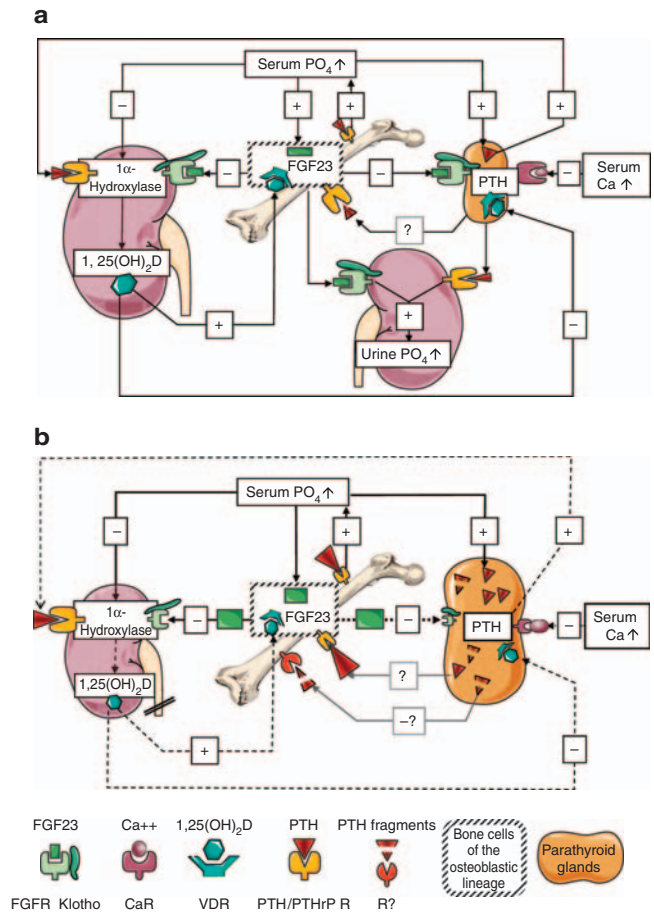

Figure 1 | Regulation and effects of FGF23 on phosphate metabolism. Regulation and metabolic effects of FGF23 on phosphate (PO_4) metabolism in subjects with normal renal function (**a**) and in patients with chronic kidney disease on dialysis (**b**). The increase in size of FGF23 and parathyroid hormone (PTH) symbols illustrates the elevation of their serum levels. The decrease in size of FGF receptor (FGFR), vitamin D receptor (VDR), and calcium receptor (CaR) symbols reflects the reduction of protein expression. Thick arrows denote a strong effect; dotted lines mean an attenuated pathway. In (**b**): note enlarged parathyroid glands and decreased kidney mass. Question marks illustrate some of the remaining questions: potential effect of PTH on FGF23 release; potential receptor for PTH fragments. The decrease in Klotho and FGFR1 expression in parathyroid glands blunts the negative effects of FGF23 on PTH secretion. 1,25$(OH)_2$D, 1,25$(OH)_2$ vitamin D; R, receptor; PTHrP, PTH-related protein.

suggesting that Klotho may regulate PTH secretion despite high FGF23 levels. In addition, Brown *et al.* described the case of a patient with Jansen's disease—which is related to a constitutively activated PTH/PTH-related protein receptor and thus mimics the features of primary hyperparathyroidism—who had elevated serum levels of FGF23. These data suggest that constitutive activation of the PTH/PTH-related protein signaling pathway may have, directly or indirectly, a stimulatory effect on bone-cell FGF23 release,[12] despite hypophosphatemia. Second, in chronic renal failure, the progressive elevation of serum phosphate induces a progressive increase in FGF23 release as glomerular filtration declines, resulting in very high serum levels in CKD5d patients. Elevated FGF23 contributes to the reduction of serum calcitriol and therefore plays a role in the development of secondary hyperparathyroidism (II HPT),[13] actually predicting its severity[14] (Figure 1b).

In a recent issue of *Kidney International*, Komaba *et al.*,[15] using immunohistochemistry, showed that expression of both FGFR1 and Klotho proteins was reduced in hyperplastic parathyroid glands from 25 CKD5d patients as compared with five normal parathyroid tissues. Klotho and FGFR1 expression was even lower in glands with nodular hyperplasia as compared with diffuse hyperplasia, which coincided with higher nuclear labeling of Ki67, a marker of cell proliferation. These data suggest that the expected effects of high serum FGF23 concentrations on PTH secretion could be blunted by a decreased sensitivity of hyperplastic parathyroid glands to FGF23, through the downregulation of its receptor and co-receptor. Interestingly, Klotho expression was shown to be also reduced in the kidneys of CKD patients.[16]

In line with the work of Komaba *et al.*[15] in humans, Galitzer *et al.* report a similar decrease in both protein and mRNA expression of FGFR1 and Klotho in parathyroid glands of rats with advanced CKD.[17] Moreover, after 6 weeks of renal failure, FGF23 administration fails to reduce PTH serum levels or to induce *egr1* parathyroid gland expression *in vivo* as well as PTH mRNA and protein expression *ex vivo*.

Similar mechanisms of refractory II HPT have been previously described. Indeed, the decrease in VDR and calcium receptor (CaR) expression (more marked in the nodular zones) in parathyroid glands of uremic rodents and humans (also mentioned in the work of Galitzer *et al.*, [17]) was reported earlier, potentially explaining the resistance of parathyroid glands to calcitriol therapy or high serum calcium.[18,19] However, despite a number of studies in animal models of CKD, the respective role and hierarchy of the loss of VDR and CaR, and now of FGFR1, in the genesis of refractory secondary hyperparathyroidism and parathyroid gland hyperplasia have not been fully unraveled. Several issues remain to be addressed in order to further understand the pathophysiological meaning of these findings. Is there one common CKD-induced mechanism (hyperphosphatemia, for instance) that leads to the decline of these receptors' (and co-receptors') expression, or are they interrelated? When does the lowering of FGFR1 and Klotho expression occur in humans? In CKD, is it a major cause of II HPT or of hyperplasia onset, or does it happen secondarily, after the decrease of VDR and CaR? In the paper by Galitzer *et al.*,[17] it is shown that FGFR1 expression declines very early, before the

onset of parathyroid-cell proliferation and reduction of VDR and CaR content, whereas Klotho expression, after an early transient increase, drops down at the later stage. These data suggest, first, a predominant role of the downregulation of the FGF23 signaling pathway in the genesis of uremic parathyroid gland disorders, and second, that there might be, at the early stages of CKD, an upregulation of Klotho in order to compensate for the progressive loss of FGFR1. It was shown that both calcitriol and calcimimetics are able to increase VDR and CaR expression *in vitro* and in uremic rat models of II HPT.[20–23] How do current therapies affect FGFR1 and Klotho expression in parathyroid glands, given that in the work of Komaba *et al.*,[15] 43% of patients had received cinacalcet treatment before surgery? Are members of the antidiabetes glitazone family, agonists of PPARγ, a factor that stimulates Klotho transcription,[24] potentially useful in the treatment of II HPT by restoring Klotho expression in parathyroid glands? Further use of animal models of renal insufficiency–induced II HPT, as in the work of Galitzer *et al.*,[17] will help us to better understand the observations of Komaba *et al.*[15] and the kinetics of these disorders; meanwhile, data obtained from human parathyroid glands, especially with nodular hyperplasia, remain necessary, since the nodular feature of this disorder cannot be fully mimicked in animals.[25]

DISCLOSURE
The author declared no competing interests.

REFERENCES

1. White KE, Evans WE, O'Riordan JLH *et al.* Autosomal dominant hypophosphataemic rickets is associated with mutations in *FGF23*. *Nat Genet* 2000; **26**: 345–348.
2. Liu S, Zhou J, Tang W *et al.* Pathogenic role of Fgf23 in Hyp mice. *Am J Physiol Endocrinol Metab* 2006; **291**: E38–E49.
3. Shimada T, Hasegawa H, Yamazaki Y *et al.* FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res* 2004; **19**: 429–435.
4. Sitara D, Razzaque MS, St-Arnaud R *et al.* Genetic ablation of vitamin D activation pathway reverses biochemical and skeletal anomalies in Fgf-23-null animals. *Am J Pathol* 2006; **169**: 2161–2170.
5. Ohnishi M, Nakatani T, Lanske B *et al.* Reversal of mineral ion homeostasis and soft-tissue calcification of klotho knockout mice by deletion of vitamin D 1alpha-hydroxylase. *Kidney Int* 2009; **75**: 1166–1172.
6. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V *et al.* The parathyroid is a target organ for FGF23 in rats. *J Clin Invest* 2007; **117**: 4003–4008.
7. Urakawa I, Yamazaki Y, Shimada T *et al.* Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* 2006; **444**: 770–774.
8. Krajisnik T, Björklund P, Marsell R *et al.* FGF-23 regulates parathyroid hormone and 1α-hydroxylase expression in cultured bovine parathyroid cells. *J Endocrinol* 2007; **195**: 125–131.
9. Imura A, Tsuji Y, Murata M *et al.* α-Klotho as a regulator of calcium homeostasis. *Science* 2007; **316**: 1615–1618.
10. Bai X, Miao D, Li J *et al.* Transgenic mice overexpressing human fibroblast growth factor 23 (R176Q) delineate a putative role for parathyroid hormone in renal phosphate wasting disorders. *Endocrinology* 2004; **145**: 5269–5279.
11. Brownstein CA, Adler F, Nelson-Williams C *et al.* A translocation causing increased alpha-klotho level results in hypophosphatemic rickets and hyperparathyroidism. *Proc Natl Acad Sci USA* 2008; **105**: 3455–3460.
12. Brown WW, Jüppner H, Langman CB *et al.* Hypophosphatemia with elevations in serum fibroblast growth factor 23 in a child with Jansen's metaphyseal chondrodysplasia. *J Clin Endocrinol Metab* 2009; **94**: 17–20.
13. Shigematsu T, Kazama JJ, Yamashita T *et al.* Possible involvement of circulating fibroblast growth factor 23 in the development of secondary hyperparathyroidism associated with renal insufficiency. *Am J Kidney Dis* 2004; **44**: 250–256.
14. Nakanishi S, Kazama JJ, Nii-Kono T *et al.* Serum fibroblast growth factor-23 levels predict the future refractory hyperparathyroidism in dialysis patients. *Kidney Int* 2005; **67**: 1171–1178.
15. Komaba H, Goto S, Fujii H *et al.* Depressed expression of Klotho and FGF receptor 1 in hyperplastic parathyroid glands from uremic patients. *Kidney Int* 2010; **77**: 232–238.
16. Koh N, Fujimori T, Nishiguchi S *et al.* Severely reduced production of klotho in human chronic renal failure kidney. *Biochem Biophys Res Commun* 2001; **280**: 1015–1020.
17. Galitzer H, Ben-Dov IZ, Silver J *et al.* Parathyroid cell resistance to fibroblast growth factor 23 in secondary hyperparathyroidism of chronic kidney disease. *Kidney Int* 2010; **77**: 211–218.
18. Gogusev J, Duchambon P, Hory B *et al.* Depressed expression of calcium receptor in parathyroid gland tissue of patients with hyperparathyroidism. *Kidney Int* 1997; **51**: 328–336.
19. Brown AJ, Dusso A, López-Hilker S *et al.* 1,25-(OH)2D receptors are decreased in parathyroid glands from chronically uremic dogs. *Kidney Int* 1989; **35**: 19–23.
20. Canaff L, Hendy GN. Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. *J Biol Chem* 2002; **277**: 30337–30350.
21. Carrillo-Lopez N, Alvarez-Hernandez D, Gonzalez-Suarez I *et al.* Simultaneous changes in the calcium-sensing receptor and the vitamin D receptor under the influence of calcium and calcitriol. *Nephrol Dial Transplant* 2008; **23**: 3479–3484.
22. Mizobuchi M, Hatamura I, Ogata H *et al.* Calcimimetic compound upregulates decreased calcium-sensing receptor expression level in parathyroid glands of rats with chronic renal insufficiency. *J Am Soc Nephrol* 2004; **15**: 2579–2587.
23. Rodriguez ME, Almaden Y, Cañadillas S *et al.* The calcimimetic R-568 increases vitamin D receptor expression in rat parathyroid glands. *Am J Physiol Renal Physiol* 2007; **292**: F1390–F1395.
24. Yang HC, Deleuze S, Zuo Y *et al.* The PPARgamma agonist pioglitazone ameliorates aging-related progressive renal injury. *J Am Soc Nephrol* 2009; **20**: 2380–2388.
25. Olgaard K, Lewin E. Can hyperparathyroid bone disease be arrested or reversed? *Clin J Am Soc Nephrol* 2006; **1**: 367–373.